BMNR 344
1423

Relation of Spittle Insect Feeding to the Dying of Red Pine and Jack Pine in the Lake States

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1/ This report contains the results of a cooperative study in which many agencies are interested. These include the Bureau of Entomology and Plant Quarantine and the Bureau of Plant Industry, Soils, and Agricultural Engineering both of the Agricultural Research Administration, and the Forest Service, in the United States Department of Agriculture; the Department of Economic Entomology and the Department of Plant Pathology both of the University of Wisconsin; and the Wisconsin Conservation Commission. The writer acknowledges the great interest shown and the many helpful suggestions made by the many individuals in these various organizations. These include F. C. Craighead, H. J. MacAloney, H. C. Secrest, A. J. Riker, C. L. Fluke, R. H. Gruenhagen, L. M. Hutchins and C. A. Richards.

Since 1941 severe injury has occurred to red pine, Pinus resinosa Ait., and jack pine, P. banksiana Lamb., plantations in many parts of the coniferous forest region of the Lake States. In some places naturally established usually open grown stands have also been injured. Twigs, tops, branches and whole trees have died with a typical top to bottom progression resulting in severe damage on areas as large as 150 to 200 acres. During the years 1941 through 1943 Secrest (1944) studied this injury in Michigan after which he concluded that a spittle insect, Aphrophora saratogensis (Fitch), was the causal agent. He suggested that the injury to the trees was caused primarily by two factors. These were the removal of sap by the insect, and the girdling effect produced by the infiltration of resin into the injured tissues around feeding punctures and the subsequent resin blocking of the conducting tissues. Last year (1943), however, A. J. Riker, Plant Pathologist at the University of Wisconsin, examined the dying trees and from the margins of the living and necrotic areas of the inner bark he was able to isolate a fungus. This finding introduced the possibility that more than one organism may be involved; therefore, further study on the etiology was desirable. As a result more studies were carried out during 1944 in northern Wisconsin where R. H. Gruenhagen conducted the mycological pathology studies while I was assigned to deal with the entomological aspects. The results of my work on the etiology are presented in this paper.

Nature of Insect Feeding Injury. -- Only the adult insects fed on pine. The nymphs formed their spittle masses and fed in the vicinity of the root collars on many species of low growing woody and herbaceous plants. Transformation of the nymphs to adults began on July 4 and continued thereafter for a period of one month; consequently the insect in the pine feeding stage was abundant throughout the months of July and August.

When feeding on pine the insects located themselves on the needle bearing branches which, as the data in table 1 show, were the parts most frequently selected for feeding. Considering the whole feeding period the attacks were heavier on the previous year's growth of the branches than on the current season's shoots. This, however, was true only for the trees in the area where the insects were extremely abundant. In the areas where fewer insects were present the averages were 1.3 and 1.4 punctures per square cm. (47 observations) for the current and the year old branches respectively. There even was an indication that early in the feeding period the current season's shoots were preferred. The top branches of the trees were slightly more desirable than those close to the ground (4.9 versus 3.8 punctures per square cm. respectively for 4 paired observations).

Table 1. -- Location of attacks on jack pine by Aphrophora saratogensis

Age of Branches	Diameter	Feeding Punctures per Square Cm.	Area Examined
Years	Inches	Number	Sq. Cm.
Current	0.3	7.6	338
1	.5	9.7	545
2	.8	3.3	271
3	1.2	1.0	105
5	1.6	0.1	200
7	1.9	0	121

The above data as well as most of that which follows are for jack pine. Time was not available to study intensively both red pine and jack pine, however, it was found that when the two species were located together the red pine was more heavily attacked. For 2 paired observations with 10 trees sampled for each the results were 5.5 and 1.9 punctures per square cm. for red pine and jack pine respectively.

Shortly after the stylets had been withdrawn the feeding punctures could be detected and easily followed in the inner bark by means of a discoloration which delimited the tiny punctures. Aided by this characteristic it was found that the stylets were inserted directly into the phloem tissue and stopped at the inner bark-xylem interface. Sometimes, however, punctures were observed that passed into the phloem tangentially to the xylem. Necrosis occurred first (after 24 to 48 hours) to the tissue in the center of the phloem. Simultaneously, the tissue in the cambial region entally to that decomposing became visibly different from that adjacent to uninjured areas. It had a silvery opaqueness and, when stained, the cells accumulated the dye, neutral red, more rapidly than adjacent cells. As each puncture aged it became in-

creasingly difficult to separate the injured inner bark from the xylem indicating that, even though the cambial cells were still alive, they had stopped proliferating. The necrosis progressed slowly, reaching the xylem in about two weeks. These observations suggest that death of the cambial tissue was the result of starvation due to the previous destruction of the adjacent food supplying phloem tissue, and possibly abetted by the rapid infiltration of resin which followed the tissue destruction. The resin was supplied by the single row of canals in the center of the inner bark tissues. These extended longitudinally and were spaced 1 to 2 mm. apart. Sometimes when pressure was applied around the termini of recently formed punctures droplets of resin appeared on the ental surface of the inner bark.

Subsequently, if the attack had not been too severe, the surrounding uninjured cambial cells divided tangentially and longitudinally as well as radially and eventually repaired the injury leaving, however, a small pitch pocket in the wood.

The area of inner bark destroyed as the result of each puncture averaged 3.1 square mm. (200 observations, range 0 to 8 square mm.).

The initial breakdown of the phloem tissue appears to be due to salivary toxins injected while feeding plus, possibly, the dehydration effect resulting from the withdrawal of sap. Observations on the first factor were made by macerating excised salivary glands on the inner bark tissue. After 12 to 24 hours exposure the surface cells were dead. They would not accumulate the dye, neutral red, and the tissue became soft and disintegrated. When salivary glands were first

heated they did not produce this effect; therefore, the substance responsible was either of an enzymatic nature or else due to associated microorganisms. With regard to this latter suggestion, however, when tiny chips of necrotic tissue were obtained from the margins between living and necrotic areas of the inner bark of dying trees and placed on healthy inner bark they did not produce necrosis during the short period of the tests (2 days).

Effect of Feeding Injury on Host Moisture Relations. -- Studies of the feeding habits revealed that Aphrophora saratogensis was extremely voracious hence very capable of causing injury. Observations on this aspect were made indirectly by measuring the quantity of liquid excreted by the insects. They were caged either on twigs in small heavily waxed paper boxes (2 1/2 cubic inches) or on branches placed in larger boxes (1 cubic foot) the bottoms of which were covered with wax paper. At the end of each 24-hour period the excreted liquid could be easily picked up and measured with the aid of a pipette. In calculating the results a correction was made for evaporation. This was computed from data collected on the rate of evaporation that occurred in check boxes which were similar in every respect to the other boxes except that no insects were added. Excreted liquid obtained from the previous day's tests was used for the evaporating liquid.

Using the above described technique it was found that each insect withdrew, or more correctly excreted, during each 24-hour period an average of .33 cc. of liquid which in turn had been obtained from an average of 1.04 punctures. The range was 0 to 1.54 for the 53 obser-

vations. In most of the tests the insects were allowed to feed on branches placed in bottles of water, however, for 8 of the observations the small paper boxes were attached to trees in the field. For these the average amount of liquid exuded was .25 cc., no correction being made for evaporation. If 30 percent (average amount of evaporation for the other tests) is added for evaporation the averages for the cut branches and the field tests are the same. With these data and knowing that the inner bark is 1 to 2 mm. thick and contains 213 percent moisture (25 observations) the amount of liquid removed as the result of each puncture was more than that contained in over 1 square cm. of inner bark - a very considerable amount.

When the needles on the trees heavily fed upon became chlorotic early in August it was suspected that the terminal needle bearing twigs were suffering from a moisture deficiency. Later this suspicion was verified when moisture determinations were made. Both the current season shoots and the year old branches from trees heavily attacked in 1944 contained less moisture than similar sized branches collected from trees growing in an area where the insects were less abundant. For the current season's twigs the moisture content was 116 percent (dry weight basis) as compared with 155 percent for the uninjured trees, while for the year old branches the values were 107 and 129 percent respectively. Four observations were made on the current season's growth while only two were made on the one year branches. Needles, needle sheaths and terminal buds were removed before weighing and drying.

During mid-September a more complete study was made on this relation of feeding punctures to the moisture deficiency of the lateral terminals. The results presented in table 2 show that there was a direct relation between the number of feeding punctures and the moisture deficiency.

Table 2.-- Relation of Aphrophora saratogensis feeding puncture concentration to moisture content of current season shoots of jack pine.

Punctures per Square Cm.	Moisture Content of Shoots	Inner Bark Destroyed	Observations	Condition
Number	Percent	Percent	Number	
0.4	167	1	7	Green
2.8	143	8	6	"
8.3	134	25	11	Chlorotic
12.8	118	38	13	Dying
17.1	101	51	5	"
21.2	85	63	1	"
----	77	--	3	Dead

I am not prepared to say how much of this dehydration should be attributed to sap withdrawal and how much to the reduction in conductivity as the result of resin infiltration into the water conducting tissues. Nevertheless, a few data were collected on both aspects.

For determining the effect of sap withdrawal one pair of equal sized branches was collected from each of 3 trees and immediately placed in water. The following day insects were introduced and allowed to feed on one branch of each pair for a period of 7 days. At the end of this period the punctures were counted and the moisture content of the twigs determined. During the 7-day period the insects made an

average of 69.7 punctures per shoot and in so doing excreted an average of 25 cc. of liquid. At the end of the feeding period the shoot moisture content of these twigs averaged 199 percent as compared with 219 percent for the checks. Even though an average of 67 percent of the total moisture content of the twigs was removed each day by the insects the moisture deficit at the end of the test was only 9 percent. This indicates that for pine branches standing in water any moisture withdrawn by insects is quickly replenished. The food materials contained in the liquid extracted, however, would be lost to the plant forever.

It is unfortunate that the above test was not checked on branches intact on the trees. Nevertheless, subsequent observations showed that resin infiltration of the conducting tissue resulting from the feeding injury was the more important factor. In these tests branch segments 5 cm. long were attached to rubber tubing through which water was introduced and maintained at a constant pressure of 1.95 pounds per square inch. This was done by using the syphon principle with a water head of 4 1/2 feet. After precautions had been taken to eliminate air from the system for each test it was allowed to run for a period of 24 hours during which time the water passing through the wood was collected. The results obtained (table 3) show that there was an inverse relation between the number of punctures and the conductivity.

Table 3.-- Conductive capacity of year old branches as affected by *Aphrophora saratogensis* feeding punctures.

Feeding Punctures per Square Cm.	Conduction per 24 Hours	Observations
Number	C. C.	Numbers
0.4	46.7	12
2.3	34.8	14
7.6	8.1	8
12.2	1.2	10

A corollary observation on this aspect was made by constructing simple potometers by inserting branches in small mouthed jars containing a known quantity of water. Eleven tests were made on paired branches of equal size - one had been heavily fed upon while the corresponding check was only lightly injured. The heavily attacked branches averaging 8.4 punctures per square cm. consumed an average of 3.4 cc. every 24 hours while the check branches containing only 2.4 punctures per square cm. used 12.3 cc. per 24 hours. It is obvious that the water used by the check branches would not be delivered if this liquid had to pass through sections severely injured by feeding punctures. See table 3.

These results suggest that resin infiltration following feeding by the spittle insect was the most important factor causing dehydration of the needle bearing twigs and branches.

The minimum moisture content threshold for jack pine was not determined nor could it be found in the literature. Marshall (1931), however, has presented data of this type for seedlings of 5 species of native pine as well as for a few other species of conifers. The

threshold values he obtained for the several species of pine varied from 59 to 87 percent of the moisture content of healthy plants. In the present study the current season's needles and shoots had hardened before dying commenced in late summer and autumn; therefore, neither the needles nor the shoots visibly wilted previous to dying. Yellow needle color, therefore, was the first visible external sign that the life threshold point had been reached while internally, necrosis of the inner bark was starting. Frequently this dying of the inner bark started in the year old branches, where the insect feeding puncture concentration was heaviest (table 1), then progressed both basally and distally. The necrosis started in a few places and was characterized by coalescing of the numerous small (3 sq. mm.) dead spots resulting from spittle insect feeding. The moisture content of the current year's shoots collected from branches in which a few of these spreading spots were present was found to average 104 percent (range 88 to 129 percent for the 14 observations). The insect feeding puncture concentration averaged 14.8 (range 9.2 to 21.2). When these moisture content data are expressed as percentages of the average value found for the shoots least injured by the insects, so as to make them comparable to Marshall's data, the values range from 51 to 77 percent with an average of 63. These values are reasonable when compared with those Marshall (1931) obtained for other species of pine, especially when it is considered that he used the more succulent seedling stage and included the needles in determining the moisture contents. Nevertheless, the results of the present work indicate that death of the jack pine branches began only after the moisture content had decreased to a very low level.

As would be expected the trees on drouthy poor sites were most severely injured. This was especially noticeable on the area studied for here good and poor sites were adjacent. In the numerous depressions present in the area (1 to 2 acres in size and 20 to 30 feet below the surrounding elevation) the site was good hence vigorous growing trees were present in dense stands while the opposite was true for the rim areas around these holes. The larger trees were more densely foliaged and more densely spaced, all the result of the better site, hence the concentration of insect feeding punctures was not dense enough to cause branch killing as the result of the 1943 attack. During the present season, however, injury has occurred to trees further down the slopes and in the shallower depressions and more injury is expected to show up during the coming winter. This season the dehydration and subsequent dying of the tops and branches was probably accentuated by the rather dry period during July and August (total rainfall departure for the two months was minus 3.7 inches or 49 percent) although this should have been partly offset by the excess during May and June (3.2 inches or 43 percent).

Relation of Insect Population Density to Injury.-- When the problem was analyzed from the aspect of insect abundance several significant observations were made. During the last few years in several widely separated areas where injury has occurred Aphrophora saratogensis has always been present in large numbers preceding the appearance of the dying trees. Secrest (1944) reported that this occurred in the area he studied in Michigan. The writer observed the same situation in Minnesota and later in Wisconsin. Last season, 1944, however, was

the first time insect population counts were made. This was studied in the area where the injury was severe and compared with similar data collected in other nearby uninjured plantations. The latter areas were not free of the spittle insect but the trees had suffered only light twig flagging.

As the nymphal population data presented in table 4 were collected only during the last stadium when most of the insects were on sweet fern, Comptonia peregrina (L.), the population density was computed solely from counts made on this species. The other desired hosts at this time were Salix and Rubus but these were not very abundant. The adult population was determined by counting the insects observed on the trees during a 5-minute interval. During this period of time the branches of four 7-foot trees could be examined. The data collected and presented in table 4 support the previous observations that injury was most prevalent where the spittle insects were most numerous.

Table 4.-- Relation between abundance of Aphrophora saratogensis and the severity of injury to jack pine.

Trees Severely Injured and in Poor Condition	Nymphal Population per Square Milacre	Stems Observed	Adult Insects Observed per 5-minute Period	Observations	Feeding Punctures per Square Cm.	Observations
Percent	Number	Number	Number	Number	Number	Number
62	154.2	439	45.4	65	9.3	15
0-*	44.7	178	18.4	36	2.0	17

-* Light twig flagging but no branch killing.

To further check these observations experimentally two tests were established. One consisted of keeping the insects from attacking some of the trees which had not been injured too severely the previous year in the area of great insect abundance, while in the second experiment insects were caged on vigorous growing uninjured branches.

Two dozen trees were sprayed with a 1-percent solution of DDT (2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane) just before the adult spittle insects emerged. During the following two months population counts were made on these trees every day or two. Check counts were made at the same time on the adjacent unsprayed trees. The puncture counts and moisture determinations were made the latter part of September. The results obtained (table 5) showed that DDT was effective in keeping the trees relatively free of insects, hence no injury occurred to these sprayed trees during the current year even though they had been injured in various degrees the previous year.

Table 5.— Protection of jack pine from spittle insect attack by spraying with DDT.

Treatment	Insects Observed per 5-minute Period	Observations	Feeding Punctures per Square Cm.	Observations	Moisture Content 1944 Shoot	Observations	Trees Severely Injured
	Number	Number	Number	Number	Percent	Number	Percent
Sprayed	3.0	43	0.3	10	169	5	0
Unsprayed	38.3	43	8.2	10	122	5	62

These trees which were protected from insect attack during 1944 by spraying with DDT had an average of 20.4 lineal feet of dead branches and twigs per tree as a result of the 1943 attack. During last autumn these dead parts were covered with numerous perithecia of the fungus thought by the plant pathologists to be implicated in the death of the trees. Thus the fungus was present in these dead branches and in contact with the living portions of the trees yet in no case did more injury show up on these treated trees during last summer and autumn. On the untreated trees, however, current injury was prevalent (table 5).

The corollary to this experiment consisted of caging different numbers of insects on uninjured vigorous growing branches. The insects were reared in cages during the last few days of their nymphal life; therefore, these adults did not have a chance to feed on any pine, diseased or otherwise, before they were introduced into the cages with the healthy branches. The cages were of the sleeve type - 8 inches in diameter and 2 feet long. These were slipped over the branches and the attached cloth sleeves closed at both ends. Insect mortality was heavy in these small cages. After one week 12, 22, 32, 39 and 41 percent of the insects were dead in the cages which originally contained 10, 30, 60, 100 and 200 insects respectively; after two weeks the mortality percentages were 32, 42, 58, 75 and 75 respectively. Nevertheless, the insects lived and fed long enough to cause the injury shown in table 6. Here again there was a direct relation between the number of insects and the amount of injury.

Table 6.-- Injury to jack pine caused by different concentrations of spittle insects.

Insects	Branches and Twigs Killed per Cage	Average Branch and Twig Length per Cage	Observations
Number	Percent	Feet	Number
0	0	14.3	4
10	0.8	13.0	4
30	1.6	15.1	4
60	20.7	12.4	4
100	33.4	17.1	4
200	69.8	15.7	4

Summary.-- Every way the problem was examined from the entomological aspect the same answer appeared; namely, injury to the pine was always correlated with the severity of the spittle insect attack - the heavier the attack the more the injury. The attacks were concentrated on the needle bearing branches of the trees and these were the parts that died first. When trees in the area of great insect abundance were protected from the insect attacks by spraying with DDT they were not injured. The more spittle insects that were concentrated in cages on vigorous growing branches the more the injury to the branches. Following the tissue destruction from insect feeding resin infiltered into the conductive tissue which prevented the normal flow of water. This, in turn, caused the dehydration of the needle bearing branches. After the moisture content of the current season's shoots decreased below about 130 percent the needles yellowed and the twigs and branches died.

These findings prompt me to conclude that the spittle insect, Aphrophora saratogensis, was the primary cause of the injury investi-

gated and that the fungi present in the dying trees were secondary.

Literature Cited

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